

(FILE 'HOME' ENTERED AT 12:42:34 ON 05 JUN 2000)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICINF'  
ENTERED AT 12:43:22 ON 05 JUN 2000

L1 37246 S (RECOMBINANT OR ARTIFICIAL) AND CHROMOSOME  
L2 960 S L1 AND ENDONUCLEASE  
L3 0 S L2 AND (CSMI OR I-CSMI)  
L4 0 S L1 AND (CSMI OR I-CSMI)  
L5 42 S L1 AND (SCEI OR I-SCEI)  
L6 16 DUP REM L5 (26 DUPLICATES REMOVED)  
L7 16 SORT L6 PY  
L8 0 S L1 AND (SCEIV OR I-SCEIV)  
L9 0 S L1 AND (PANI OR I-PANI)

1, 7      14  
-6      11  
-7      12  
-9      16  
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16      2  
         4  
         5  
         (8)

L7 ANSWER 1 OF 16 MEDLINE  
TI Rapid physical mapping of YAC inserts by random integration of I-Sce I sites.  
SO HUMAN MOLECULAR GENETICS, (1993 Mar) 2 (3) 265-71.  
Journal code: BRC. ISSN: 0964-6906.  
AU Colleaux L; Rougeulle C; Avner P; Dujon B  
AB We have developed a novel strategy, based on the random insertion by homologous recombination of **artificial** I-Sce I sites within mammalian repetitive DNA sequences, which should greatly facilitate the high resolution physical mapping of large DNA fragments cloned in YAC. A set of transgenic yeast strains containing appropriately spaced I-Sce I sites within the YAC insert defines a series of nested physical intervals against which new genes, clones or DNA fragments can be mapped by simple hybridisation. Sequential hybridisation using such a series of nested YAC fragments as probes can also allow the rapid sorting of phage or cosmid libraries into contigs. This approach, which has been applied to a YAC containing a 460 kb insert from the mouse X chromosome, may also have applications for the restriction mapping of large genomic segments, mapping of exons and the search for homologous genes.

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## L7 ANSWER 2 OF 16 MEDLINE

TI Recombination initiated by double-strand breaks.

SO CURRENT GENETICS, (1993) 23 (4) 305-14.

Journal code: CUG. ISSN: 0172-8083.

AU McGill C B; Shafer B K; Derr L K; Strathern J N

AB The HO endonuclease was used to introduce a site-specific double-strand break (DSB) in an interval designed to monitor mitotic recombination. The interval included the trp1 and his3 genes inserted into chromosome III of *S. cerevisiae* between the CRY1 and MAT loci. Mitotic recombination was monitored in a diploid carrying heteroalleles of trp1 and his3. The normal recognition sites for the HO endonuclease were mutated at the MAT alleles and a synthetic recognition site for HO endonuclease was placed between trp1 and his3 on one of the chromosomes. HO-induced cleavage resulted in efficient recombination in this interval. Most of the data can be explained by double-strand gap repair in which the cut chromosome acts as the recipient. However, analysis of some of the recombinants indicates that regions of heteroduplex were generated flanking the site of the cut, and that some recombinants were the result of the cut chromosome acting as the genetic donor.

## L7 ANSWER 3 OF 16 MEDLINE

TI A novel allele of *Saccharomyces cerevisiae* RFA1 that is deficient in recombination and repair and suppressible by RAD52.

SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Mar) 15 (3) 1620-31.

Journal code: NGY. ISSN: 0270-7306.

AU Firmenich A A; Elias-Arnanz M; Berg P

AB To understand the mechanisms involved in homologous recombination, we have performed a search for *Saccharomyces cerevisiae* mutants unable to carry out plasmid-to-chromosome gene conversion. For this purpose, we have developed a colony color assay in which recombination is induced by the controlled delivery of double-strand breaks (DSBs). Recombination occurs between a chromosomal mutant ade2 allele and a second plasmid-borne ade2 allele where DSBs are introduced via the site-specific HO endonuclease. Besides isolating a number of new alleles in known rad genes, we identified a novel allele of the RFA1 gene, rfal-44, which encodes the large subunit of the heterotrimeric yeast single-stranded DNA-binding protein RPA. Characterization of rfal-44 revealed that it is, like members of the RAD52 epistasis group, sensitive to X rays, high doses of UV, and HO-induced DSBs. In addition, rfal-44 shows a reduced ability to undergo sporulation and HO-induced gene conversion. The mutation was mapped to a single-base substitution resulting in an aspartate at amino acid residue 77 instead of glycine. Moreover, all radiation sensitivities and repair defects of rfal-44 are suppressed by RAD52 in a dose-dependent manner, and one RAD52 mutant allele, rad52-34, displays nonallelic noncomplementation when crossed with rfal-44. Presented is a model accounting for this genetic interaction in which Rfa1, in a complex with Rad52, serves to assemble other proteins of the recombination-repair machinery at the site of DSBs and other kinds of DNA damage. We believe that our findings and those of J. Smith and R. Rothstein (Mol. Cell. Biol. 15:1632-1641, 1995) are the first in vivo demonstrations of the involvement of a eukaryotic single-stranded binding protein in recombination and repair processes.

## L7 ANSWER 4 OF 16 MEDLINE

TI Chromosomal double-strand break repair in Ku80-deficient cells  
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Aug 20) (17) 8929-33.  
Journal code: PV3. ISSN: 0027-8424.

AU Liang F; Romanienko P J; Weaver D T; Jeggo P A; Jasin M  
AB The x-ray sensitive hamster cell line xrs-6 is deficient in DNA double-strand break (DSB) repair and exhibits impaired V(D)J recombination. The molecular defect in this line is in the 80-kDa subunit of the Ku autoantigen, a protein that binds to DNA ends and recruits the DNA-dependent protein kinase to DNA. Using an I-SceI endonuclease expression system, chromosomal DSB repair was examined in xrs-6 and parental CHO-K1 cell lines. A DSB in chromosomal DNA increased the yield of recombinants several thousand-fold above background in both the xrs-6 and CHO-K1 cells, with recombinational repair of DSBs occurring in as many as 1 of 100 cells electroporated with the endonuclease expression vector. Thus, recombinational repair of chromosomal DSBs can occur at substantial levels in mammalian cells and it is not grossly affected in our assay by a deficiency of the Ku autoantigen. Rejoining of broken chromosome ends (end-joining) near the site of the DSB was also examined. In contrast to recombinational repair, end-joining was found to be severely impaired in the xrs-6 cells. Thus, the Ku protein appears to play a critical role in only one of the chromosomal DSB repair pathways.

L7 ANSWER 5 OF 16 MEDLINE  
TI Meiotic recombination initiated by a double-strand break in rad50 delta yeast cells otherwise unable to initiate meiotic recombination.  
SO GENETICS, (1996 Jun) 143 (2) 741-54.  
Journal code: FNH. ISSN: 0016-6731.

AU Malkova A; Ross L; Dawson D; Hoekstra M F; Haber J E  
AB Meiotic recombination in *Saccharomyces cerevisiae* is initiated by double-strand breaks (DSBs). We have developed a system to compare the properties of meiotic DSBs with those created by the site-specific HO endonuclease. HO endonuclease was expressed under the control of the meiotic-specific SPO13 promoter, creating a DSB at a single site on one of yeast's 16 chromosomes. In Rad+ strains the times of appearance of the HO-induced DSBs and of subsequent recombinants are coincident with those induced by normal meiotic DSBs. Physical monitoring of DNA showed that SPO13: HO induced gene conversions both in Rad+ and in rad50 delta cells that cannot initiate normal meiotic DSBs. We find that the RAD50 gene is important, but not essential, for recombination even after a DSB has been created in a meiotic cell. In rad50 delta cells, some DSBs are not repaired until a broken chromosome has been packaged into a spore and is subsequently germinated. This suggests that a broken chromosome does not signal an arrest of progression through meiosis. The recombination defect in rad50 delta diploids is not, however, meiotic specific, as mitotic rad50 diploids, experiencing an HO-induced DSB, exhibit similar departures from wild-type recombination.

L7 ANSWER 6 OF 16 MEDLINE  
TI A double-strand break within a yeast artificial chromosome (YAC) containing human DNA can result in YAC loss, deletion or cell lethality.  
SO MOLECULAR AND CELLULAR BIOLOGY, (1996 Aug) 16 (8) 4414-25.  
Journal code: NGY. ISSN: 0270-7306.

AU Bennett C B; Westmoreland T J; Snipe J R; Resnick M A  
AB Human chromosomal DNA contains many repeats which might provide opportunities for DNA repair. We have examined the consequences of a single double-strand break (DSB) within a 360-kb dispensable yeast artificial chromosome (YAC) containing human DNA (YAC12). An Alu-URA3-YZ sequence was targeted to several Alu sites within the YAC in strains of the yeast *Saccharomyces cerevisiae*; the strains contained a galactose-inducible HO endonuclease that cut the YAC at the YZ site. The presence of a DSB in most YACs led to deletion of the URA3 cassette, with retention of the telomeric markers, through recombination between surrounding Alus. For two YACs, the DSBs were not repaired and there was a G2 delay associated with the persistent DSBs. The presence of persistent DSBs resulted in cell death even though the YACs were dispensable. Among the survivors of the persistent DSBs, most had lost the YAC. By a pullback procedure, cell death was observed to begin at least 6 h after induction of a break. For YACs in which the DSB was rapidly repaired, the breaks did not cause cell cycle delay or lead to cell death. These results are consistent with our previous conclusion that a persistent DSB in a plasmid (YZ-CEN) also caused lethality (C. B. Bennett, A. L. Lewis, K. K. Baldwin, and M. A. Resnick, Proc. Natl. Acad. Sci. USA 90:5613-5617, 1993). However, a break in the YZ-CEN plasmid did not induce lethality in the strain (CBY) background used in the present study. The differences in survival levels appear to be due to the rapid degradation of the plasmid in the CBY strain. We, therefore, propose that for a DSB to

cause cell cycle delay and death by means other than the loss of essential genetic material, it must remain unrepaired and be long-lived

L7 ANSWER 7 OF 16 SCISEARCH COPYRIGHT 2000 ISI (R)

TI New vectors for combinatorial deletions in yeast chromosomes and for gap-repair cloning using 'split-marker' recombination

SO YEAST, (NOV 1996) Vol. 12, No. 14, pp. 1439-1458.

Publisher: JOHN WILEY & SONS LTD, BAFFINS LANE CHICHESTER, W SUSSEX, ENGLAND PO19 1UD.  
ISSN: 0749-503X.

AU Fairhead C (Reprint); Llorente B; Denis F; Soler M; Dujon B

AB New tools are needed for speedy and systematic study of the numerous genes revealed by the sequence of the yeast genome. We have developed a novel transformation strategy, based on 'split-marker' recombination, which allows generation of chromosomal deletions and direct gene cloning. For this purpose, pairs of yeast vectors have been constructed which offer a number of advantages for large-scale applications such as one-step cloning of target sequence homologs and combinatorial use. Gene deletions or gap-repair clonings are obtained by cotransformation of yeast by a pair of recombinant plasmids. Gap-repair vectors are based on the URA3 marker. Deletion vectors include the URA3, LYS2 and kanMX selection markers flanked by I-SceI sites, which allow their subsequent elimination from the transformant without the need for counter-selection. The application of the 'split-marker' vectors to the analysis of a few open reading frames of chromosome XI is described.

L7 ANSWER 8 OF 16 MEDLINE

TI Chromosomal double-strand breaks induce gene conversion at high frequency in mammalian cells.

SO MOLECULAR AND CELLULAR BIOLOGY, (1997 Nov) 17 (11) 6386-93.

Journal code: NGY. ISSN: 0270-7306.

AU Taghian D G; Nickoloff J A

AB Double-strand breaks (DSBs) stimulate chromosomal and extrachromosomal recombination and gene targeting. Transcription also stimulates spontaneous recombination by an unknown mechanism. We used *Saccharomyces cerevisiae* I-SceI to stimulate recombination between neo direct repeats in Chinese hamster ovary (CHO) cell chromosomal DNA. One neo allele was controlled by the dexamethasone-inducible mouse mammary tumor virus promoter and inactivated by an insertion containing an I-SceI site at which DSBs were introduced in vivo. The other neo allele lacked a promoter but carried 12 phenotypically silent single-base mutations that create restriction sites (restriction fragment length polymorphisms). This system allowed us to generate detailed conversion tract spectra for recipient alleles transcribed at high or low levels. Transient in vivo expression of I-SceI increased homologous recombination 2,000- to 10,000-fold, yielding recombinants at frequencies as high as 1%. Strikingly, 97% of these products arose by gene conversion. Most products had short, bidirectional conversion tracts, and in all cases, donor neo alleles (i.e., those not suffering a DSB) remained unchanged, indicating that conversion was fully nonreciprocal. DSBs in exogenous DNA are usually repaired by end joining requiring little or no homology or by nonconservative homologous recombination (single-strand annealing). In contrast, we show that chromosomal DSBs are efficiently repaired via conservative homologous recombination, principally gene conversion without associated crossing over. For DSB-induced events, similar recombination frequencies and conversion tract spectra were found under conditions of low and high transcription. Thus, transcription does not further stimulate DSB-induced recombination, nor does it appear to affect the mechanism(s) by which DSBs induce gene conversion.

L7 ANSWER 9 OF 16 MEDLINE

TI Analysis of gene targeting and intrachromosomal homologous recombination stimulated by genomic double-strand breaks in mouse embryonic stem cells.

SO MOLECULAR AND CELLULAR BIOLOGY, (1998 Jul) 18 (7) 4070-8.

Journal code: NGY. ISSN: 0270-7306.

AU Donoho G; Jasin M; Berg P

AB To investigate the effects of in vivo genomic DNA double-strand breaks on the efficiency and mechanisms of gene targeting in mouse embryonic stem cells, we have used a series of insertion and replacement vectors carrying two, one, or no genomic sites for the rare-cutting endonuclease I-SceI. These vectors were introduced into the hypoxanthine phosphoribosyltransferase (hprt) gene to produce substrates for gene-targeting (plasmid-to-chromosome) or intrachromosomal (direct repeat) homologous recombination. Recombination at the hprt locus is markedly increased following transfection with an I-SceI expression plasmid and a homologous donor plasmid (if needed). The frequency of gene targeting in clones with an I-

SceI site attains a value of 1%, 5,000-fold higher than that in clones with no I-SceI site. The use of silent restriction site polymorphisms indicates that the frequencies with which donor plasmid sequences replace the target chromosomal sequences decrease with distance from the genomic break site. The frequency of intrachromosomal recombination reaches a value of 3.1%, 120-fold higher than background spontaneous recombination. Because palindromic insertions were used as polymorphic markers, a significant number of recombinants exhibit distinct genotypic sectoring among daughter cells from a single clone, suggesting the existence of heteroduplex DNA in

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**WEST****Searches for User *skaushal* (Count = 313)**

Queries 264 through 313.










S #	Updt	Database	Query	Time	Comment
<u>S313</u>	<u>U</u>	USPT,JPAB,EPAB,DWPI	((recombinat or artificial)and chromosome ) and (I-CsmI or CsmI)	2000-06-05 12:50:01	
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<u>S310</u>	<u>U</u>	USPT,JPAB,EPAB,DWPI	((recombinat or artificial)and chromosome ) and (I-SceI or SceI)	2000-06-05 12:47:54	
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<u>S308</u>	<u>U</u>	DWPI,EPAB,JPAB,USPT	GIUDICE-G!	2000-06-01 17:34:08	
<u>S307</u>	<u>U</u>	USPT,JPAB,EPAB,DWPI	[REDACTED] (isolate)	15:26:15	
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<u>S305</u>	<u>U</u>	USPT,JPAB,EPAB,DWPI	[REDACTED] g or mouse)	2000-06-01 15:24:29	
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**WEST**[Generate Collection](#)**Search Results - Record(s) 1 through 5 of 5 returned.**☐ 1. Document ID: US 5962327 A

L5: Entry 1 of 5

File: USPT

Oct 5, 1999

US-PAT-NO: 5962327

DOCUMENT-IDENTIFIER: US 5962327 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: US 5948678 A

L5: Entry 2 of 5

File: USPT

Sep 7, 1999

US-PAT-NO: 5948678

DOCUMENT-IDENTIFIER: US 5948678 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 3. Document ID: US 5866361 A

L5: Entry 3 of 5

File: USPT

Feb 2, 1999

US-PAT-NO: 5866361

DOCUMENT-IDENTIFIER: US 5866361 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 4. Document ID: US 5792632 A

L5: Entry 4 of 5

File: USPT

Aug 11, 1998

US-PAT-NO: 5792632

DOCUMENT-IDENTIFIER: US 5792632 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 5. Document ID: US 5474896 A

L5: Entry 5 of 5

File: USPT

Dec 12, 1995

US-PAT-NO: 5474896

DOCUMENT-IDENTIFIER: US 5474896 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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Term	Documents
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I-CSMIS	0
CSMI,DWPI,EPAB,JPAB,USPT.	16
CSMIS	0
((I-CSMI OR CSMI) AND 1),USPT,JPAB,EPAB,DWPI.	5

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L5: Entry 1 of 5

File: USPT

Oct 5, 1999

US-PAT-NO: 5962327

DOCUMENT-IDENTIFIER: US 5962327 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the  
uses thereof

DATE-ISSUED: October 5, 1999

## INVENTOR-INFORMATION:

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Perrin; Arnaud	Paris	N/A	N/A	FRX
Plessis; Anne	Paris	N/A	N/A	FRX
Thierry; Agnes	Paris	N/A	N/A	FRX

US-CL-CURRENT: 435/478; 435/320.1, 536/23.2

## ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

29 Claims, 32 Drawing figures Exemplary Claim Number: 27

Number of Drawing Sheets: 24